## K562/GM-CSF VACCINATION IN COMBINATION WITH IMATINIB MESYLATE (GLEEVEC™) FOR CHRONIC MYELOID LEUKEMIA (CML)

### Technical Abstract

CML is a malignant clonal disorder of hematopoietic stem cells that affects myeloid cells, erythroid cells and platelets, leading to elevated cell counts in the blood and myeloid hyperplasia in the marrow. The genetic hallmark CML is the reciprocal translocation of chromosomes 9 and 22 forming the Philadelphia (Ph) chromosome, which results in rearrangements of regions of the *BCR* (chromosome 22) and *ABL* (chromosome 9) genes to form the hybrid *BCR-ABL* fusion gene. This translocation results in disrupted regulation of the tyrosine kinase domain of the *ABL* gene, resulting in its constitutive activation in the fusion protein. Such changes confer a proliferative and survival advantage of hematopoietic cells harboring the rearrangement, and their ultimate malignant transformation.

CML accounts for about 20 percent of newly diagnosed cases of leukemia in adults, and is most commonly identified in its chronic phase, which for years had been managed with hydroxyurea or busulfan. While these treatments are frequently successful in controlling the peripheral blood cell counts, in the absence of additional therapies, the chronic phase (typically lasting three to six years) invariably progresses to an accelerated phase followed by blast crisis, which is largely resistant to definitive therapy.

*Treatment Options-*

Chronic phase CML was among the first diseases shown to be curable by allogeneic bone marrow transplantation (BMT) in a significant percentage (70-80%) of younger patients having an HLA identical sibling. Notably, several aspects of the transplant experience in CML have firmly established CML as an immunologically responsive disease. First, an inverse correlation has been observed between the development of graft versus host disease (GVHD) and relapse from CML. Second, the relapse rate of CML patients receiving sygeneic transplants is significantly higher than in patients who receive allogeneic, HLA matched sibling donor grafts. Third, efforts to reduce the incidence and toxicity of graft versus host disease by T cell depletion of the graft led to a significant increase in leukemic relapse. Finally, the dramatic remissions induced by the infusion of donor lymphocytes in CML patients who relapse following allo-BMT, firmly established the potency of T cells in fully eradicating the malignant clone.

Whereas allogeneic BMT has been the treatment of choice for eligible CML patients for the past two decades or more, a significant fraction of these patients succumb to the toxicities of the procedure (GVHD, immunosuppression, and preparative regimen toxicity), and over two-thirds of patients either don't have a matched sibling donor or are considered too old to safely tolerate allogeneic BMT. Recent experience with non-myeloablative preparative regimens and alternate donor sources have attempted to deal with these limitations, although at present, these experimental therapies still carry a significant risk of morbidity and mortality.

In chronic phase CML, daily interferon-α can induce complete cytogenetic responses in 5 to 20% of patients and has been shown to prolong survival in such responders. Interferon-α affects proliferation and differentiation of the leukemic clone and augments immune responses against CML-associated antigens. Notably, HLA-A0201$^+$ patients achieving a complete hematologic remission (CHR) in response to interferon-α have been found to have a significant increase in the frequency of CD8$^+$ T cells specific for an HLA-A0201 restricted epitope of proteinase 3. This enzyme is a normal constituent of the myeloid granules, but has been found to be over-expressed roughly ten-fold by CML cells. The correlation between clinical and immunological responses in CML patients treated with interferon further attests to the potential for immune mediated therapies in this disease. Unfortunately the majority of CML patients on interferon-α fail to achieve complete cytogenetic remissions, and many of those who do continue to harbor persistent molecular evidence of BCR-ABL transcripts. Furthermore, side effects of interferon can be intolerable and limit long-term treatment in a substantial number of patients.

In addition to interferon, imatinib mesylate (Gleevec) is now widely considered to be front-line therapy for CML. Imatinib is a potent inhibitor of the protein tyrosine kinase of the BCR-ABL

fusion protein. The initial phase I clinical trials of imatinib revealed dramatic responses in CML patients who failed interferon–α, with 53 of 54 patients normalizing their white blood cell (WBC) count and platelet counts, usually within four weeks of initiating therapy. In this study, 54% of patients had cytogenetic responses (31% major and 13% complete), and these were demonstrable significantly earlier than with interferon. Several subsequent multi-institutional phase II trials have reported that greater than 90% of patients with interferon resistant chronic phase disease normalized their blood counts with imatinib, and nearly half had a major cytogenetic response, with complete cytogenetic responses obtained in over 40%. In the IRIS study, at a median 19 month follow-up, 95% of patients treated initially with imatinib had a complete hematologic response, and 85% had a major cytogenetic response, most of which were complete.

Despite these promising results with imatinib, resistance to this agent can occur. Some patients treated in chronic phase who initially respond to imatinib subsequently relapse with highly aggressive resistant disease. Recent reports have suggested that the primitive Ph chromosome positive CML stem cells are insensitive to imatinib *in vitro*, such that imatinib alone may be incapable of truly eradicating the disease. Furthermore, several studies examining molecular detection of BCR-ABL by RT-PCR have reported that most cytogenetic responses to imatinib, including complete responses, remain BCR/ABL positive. Indeed, an interim analysis of the IRIS study reported that only 2 of 28 patients receiving imatinib as first-line therapy reached PCR-undetectable transcript levels, and the rate of response slowed as a function of duration of therapy. Finally, of 21 patients who achieved a complete cytogenetic remission on imatinib with very low or RT-PCR-undetectable transcript levels, two-thirds had subsequent increases in tumor burden, with 19% having cytogenetic relapse, 27% having a sustained > 1 log increase in BCR/ABL transcripts, and 33% converting from undetectable to detectable disease status.

While it is somewhat difficult to develop future treatment approaches for CML given the relatively short follow-up of patients treated with imatinib and the rapid pace with which new information is forthcoming, based on the findings above, we believe it is likely that a significant fraction of CML patients will not be cured by single agent imatinib and that combination approaches are warranted, at least in subsets of patients. As highlighted above, CML has clearly been shown to be curable by T cell mediated immunity. There can be no question that the high response rate and low toxicity of imatinib mesylate makes this reagent highly attractive as a means to cytoreduce CML patients without concomitant drug-induced immunosuppression. Accordingly, the central focus of this proposal is to test the integration of imatinib with a tumor-cell based vaccine strategy that seeks to generate and/or augment a polyvalent endogenous T cell response to CML associated antigens. The investigational agent (K562/GM-CSF) is an allogeneic tumor cell line derived from a patient with CML in blast crisis which has been transfected in vitro with a plasmid DNA encoding the human cytokine, GM-CSF. K562 cells have been shown to express several of the antigens found in CML patient samples. GM-CSF transduced tumor cell based vaccines have been extensively studied in rodent models and early phase clinical trials as a means to augment T cell and antibody responses to a range of antigens expressed by the immunizing cell line.

*Objectives:*
The primary goals of this study are to see if CML patients who have been taking imatinib for greater than one year, and have achieved a complete hematologic remission and a major cytogenetic response, but still have cytogenetic or PCR based evidence of residual CML can respond to vaccination with K562/GM-CSF by mounting an immune response that can further reduce, or perhaps eliminate the residual leukemia cells. Secondary objectives are to determine if the frequency of T cells specific for four defined CML associated antigens (expressed by K562 cells) are increased following vaccination, and if so, if such responses are associated with reduction of the leukemia burden as measured by quantitative molecular techniques.

*Patient Population:*

Twenty patients with a history of chronic phase CML treated for at least one year with imatinib mesylate, who are in a complete hematologic remission, and have achieved a major cytogenetic response (<34% Ph chromosome positive cells), but still have a detectable and quantifiable CML population.

*Study Design:*
Single institution open-label trial.

*Treatment Plan*:
Patients with the above characteristics will be evaluated every six weeks for three months to establish the stability of disease burden by BCR/ABL FISH and qRT-PCR. Patients who continue to have measurable disease, with less than a one-half log further reduction in tumor burden over the three month interval, will be vaccinated with K562/GM-CSF every three weeks times four vaccination cycles. Post vaccine follow-up will occur every six weeks until 36 weeks after the initial vaccination. Yearly long-term follow up will then be performed per FDA guidelines.

*Dose:*
Each vaccine cycle will consist of $1 \times 10^8$ irradiated K562/GM-CSF cells divided into 10 intradermanl injection sites of $1 \times 10^7$ cells each.

*Endpoints:*
The primary endpoints of the trial are: 1) the measurement of the change in disease burden over time as assessed by BCR/ABL FISH and qRT-PCR, comparing the pre and post-vaccination periods, and 2) the safety of K562/GM-CSF vaccination in CML patients taking imatinib, as assessed by standard clinical and laboratory parameters. The secondary endpoint of the trial are the measurements of change in frequency of T cells specific for four CML associated antigens shown to be expressed by K562 cells (BCR/ABL p210, proteinase-3, WT-1, and survivin). These measurements will be used to test the hypothesis that vaccination with K562/GM-CSF leads to the induction or amplification of T cell responses to one or more of these candidate antigens, and that such responses correlate with a reduction in tumor burden or conversion to complete molecular remission.

Product:
K562/GM-CSF
The K562 cell line was engineered by plasmid transfection to secrete human GM-CSF. These cells are grown in suspension, irradiated at 10,000 rads to arrest cell growth, formulated in a dimethyl-sulfoxide (DMSO)-containing cryoprotectant and frozen in liquid nitrogen. K562/GM-CSF cells are thawed just prior to administration and injected intradermally without further manipulation.